

UK Patent Application (19) GB (11) 2 369 739 (13) A

(43) Date of A Publication 05.06.2002

(21) Application No 0123392.3

(22) Date of Filing 28.09.2001

(30) Priority Data

(31) 10050529

(32) 11.10.2000

(33) DE

(51) INT CL7
G02B 26/10 // H04N 1/04

(52) UK CL (Edition T)
H4F FAAL FJB F50

(56) Documents Cited
DE 019754647 A1 JP 020309881 A
US 6225628 A US 4691103 A

(58) Field of Search
UK CL (Edition T) G2J JB7WX , H4F FAAL FCCC FCCY
FJB FJC
Online: WPI, EPODOC, PAJ

(71) Applicant(s)

Leica Microsystems Heidelberg GmbH
(Incorporated in the Federal Republic of Germany)
Am Friedensplatz 3, 68165 Mannheim,
Federal Republic of Germany

(72) Inventor(s)

Johann Engelhardt
Werner Knebel

(74) Agent and/or Address for Service

Dr Walther Wolff & Co
6 Buckingham Gate, LONDON, SW1E 6JP,
United Kingdom

(54) Abstract Title

Beam control in a scanning microscope

(57) A scanning microscope comprises means (3) for acquiring and displaying a preview image (7), a microscope optical system (51) and means (5) for marking at least one region (27, 29) of interest in the preview image (7). A first beam deflection device (43, 67, 68) directs the scan field (31, 33) onto the vicinity of the region of interest; and a second beam deflection device (49, 72, 94) serves for meander-shaped scanning within the scan field (31, 33).

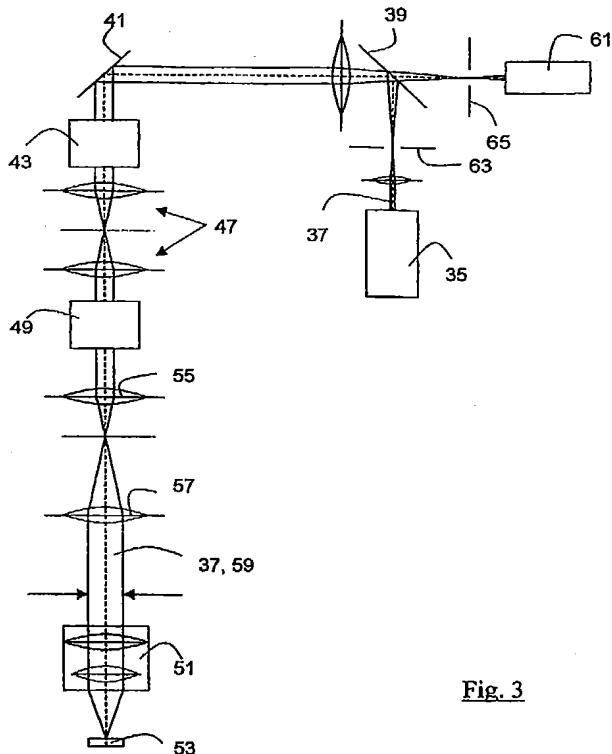


Fig. 3

GB 2 369 739 A

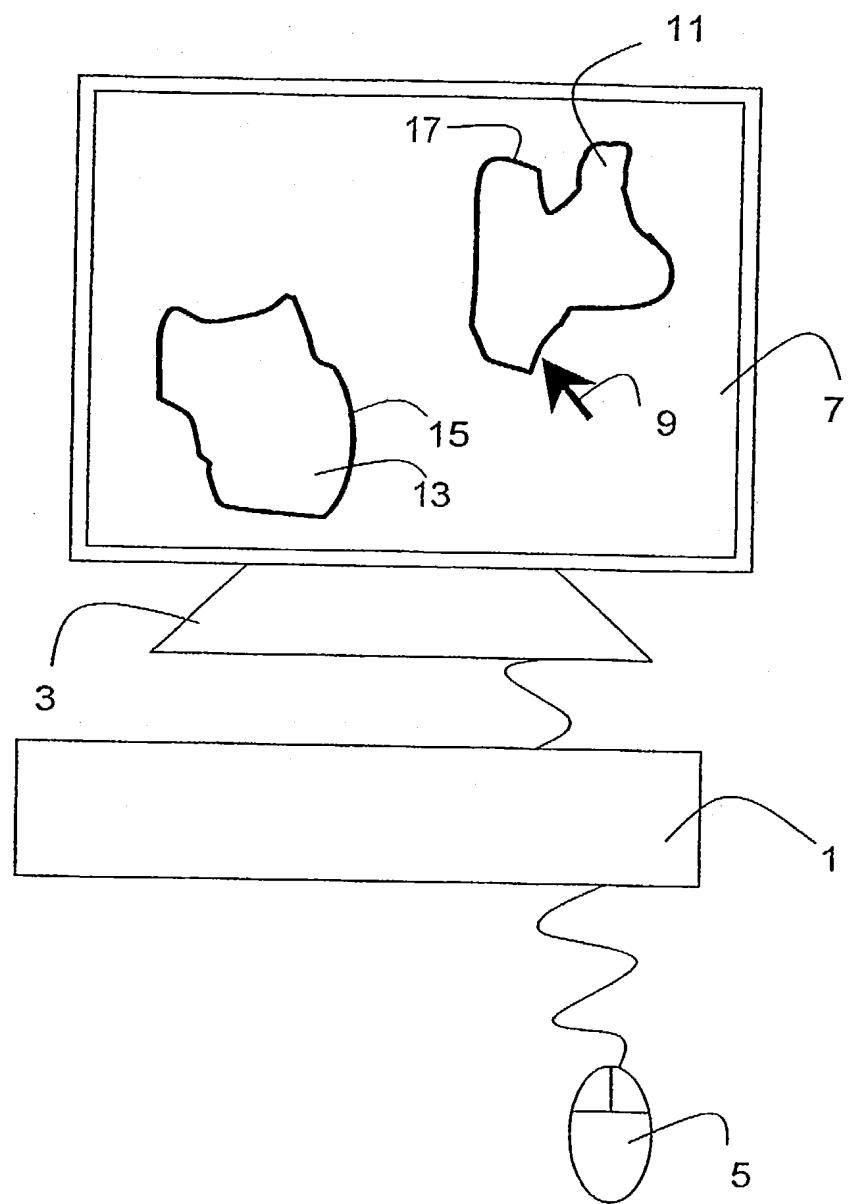


Fig. 1

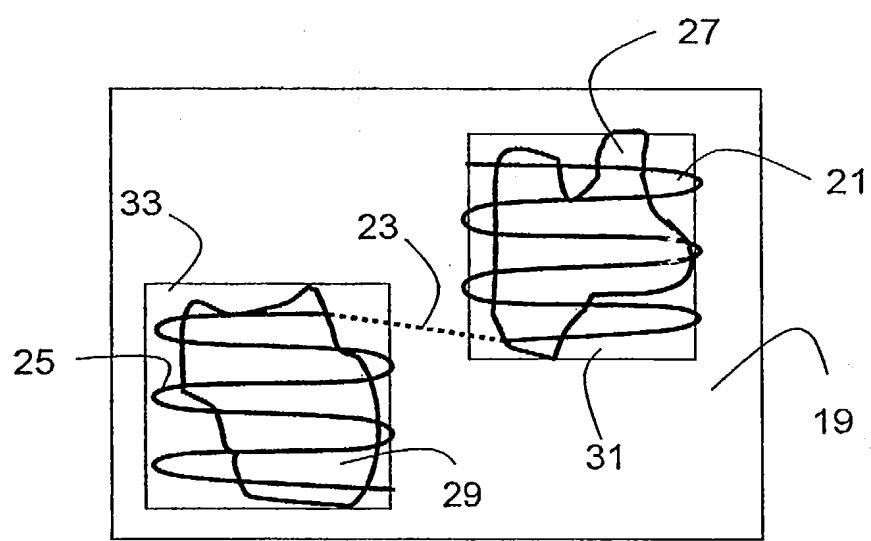


Fig. 2

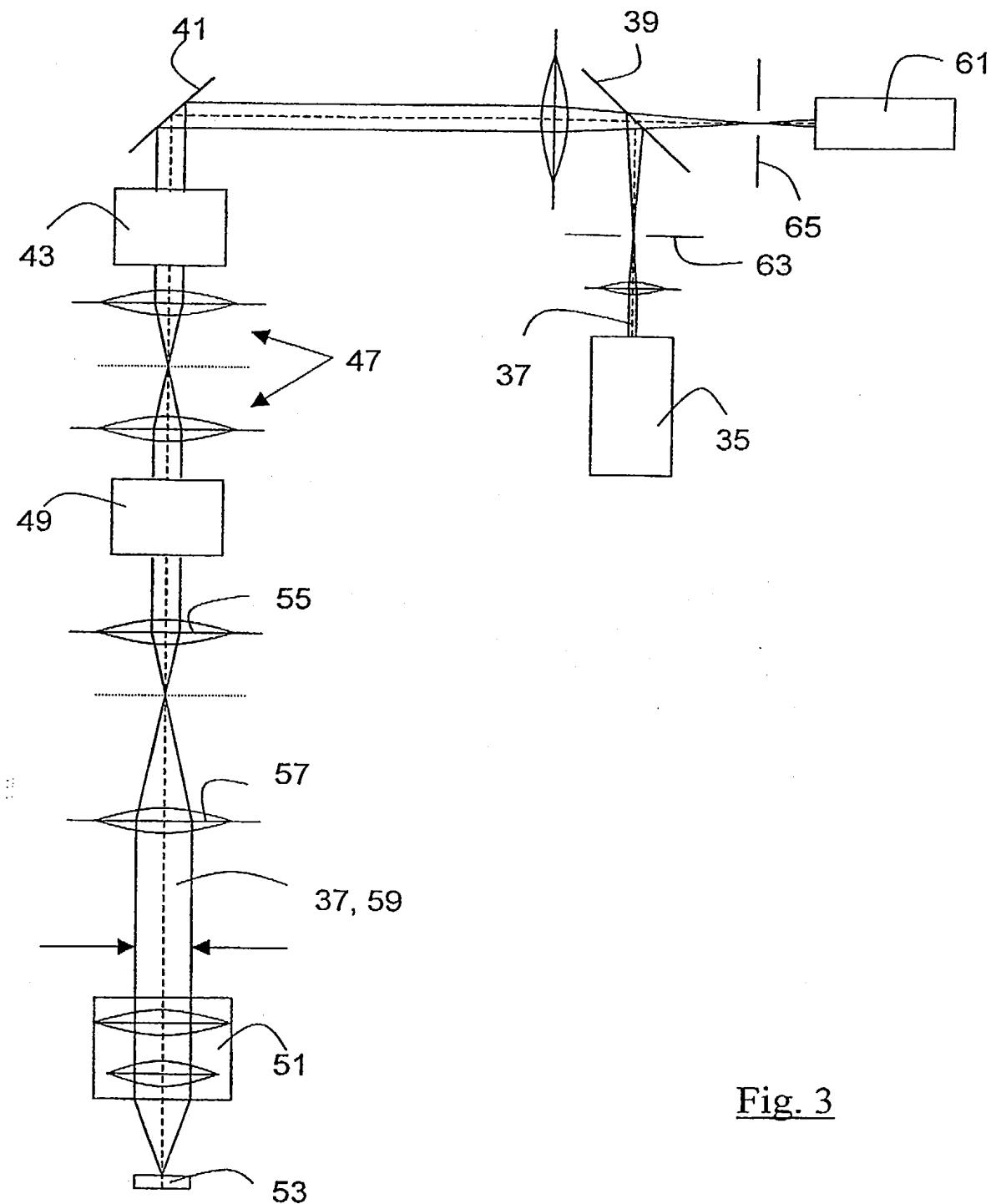


Fig. 3

4/6

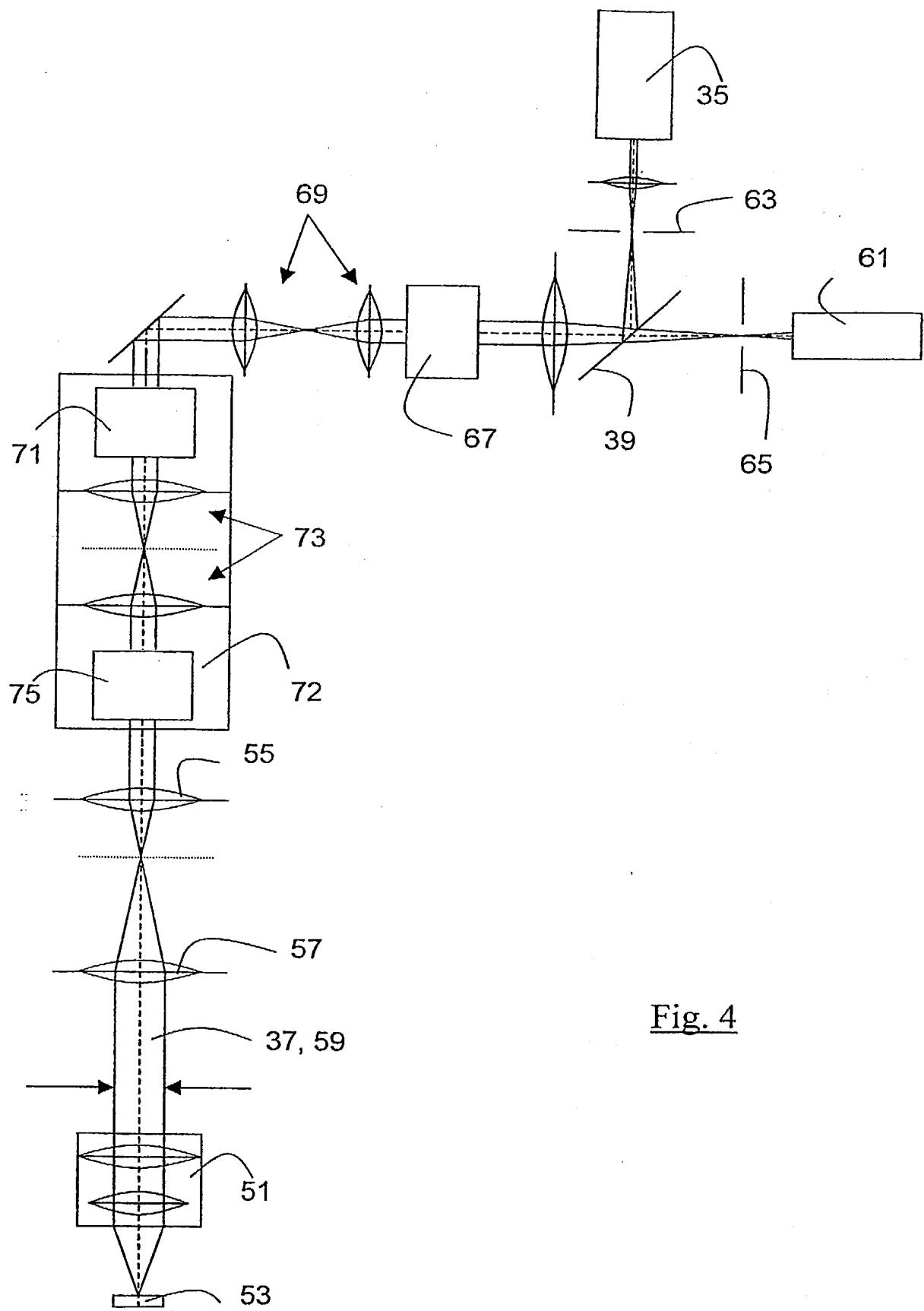


Fig. 4

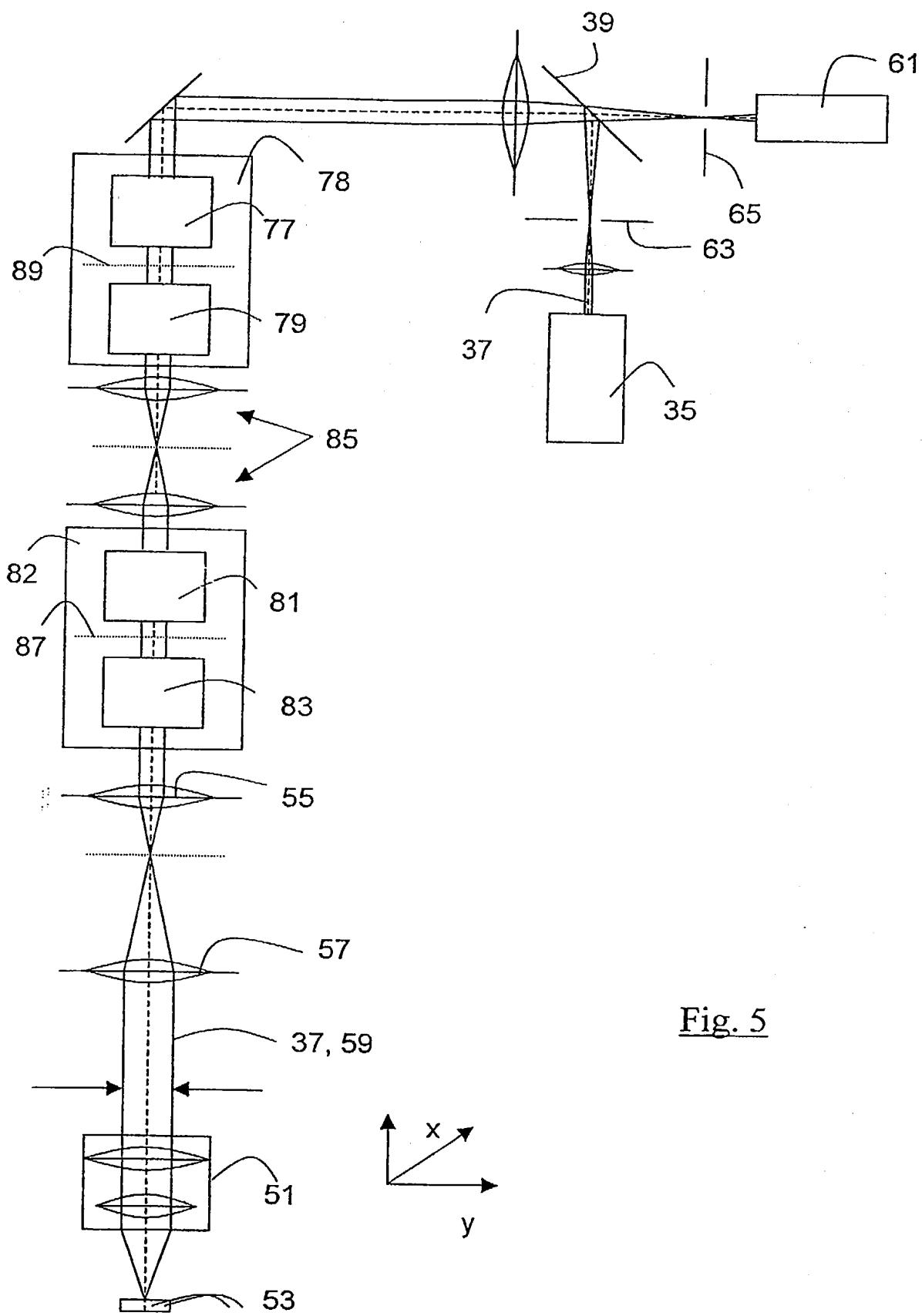
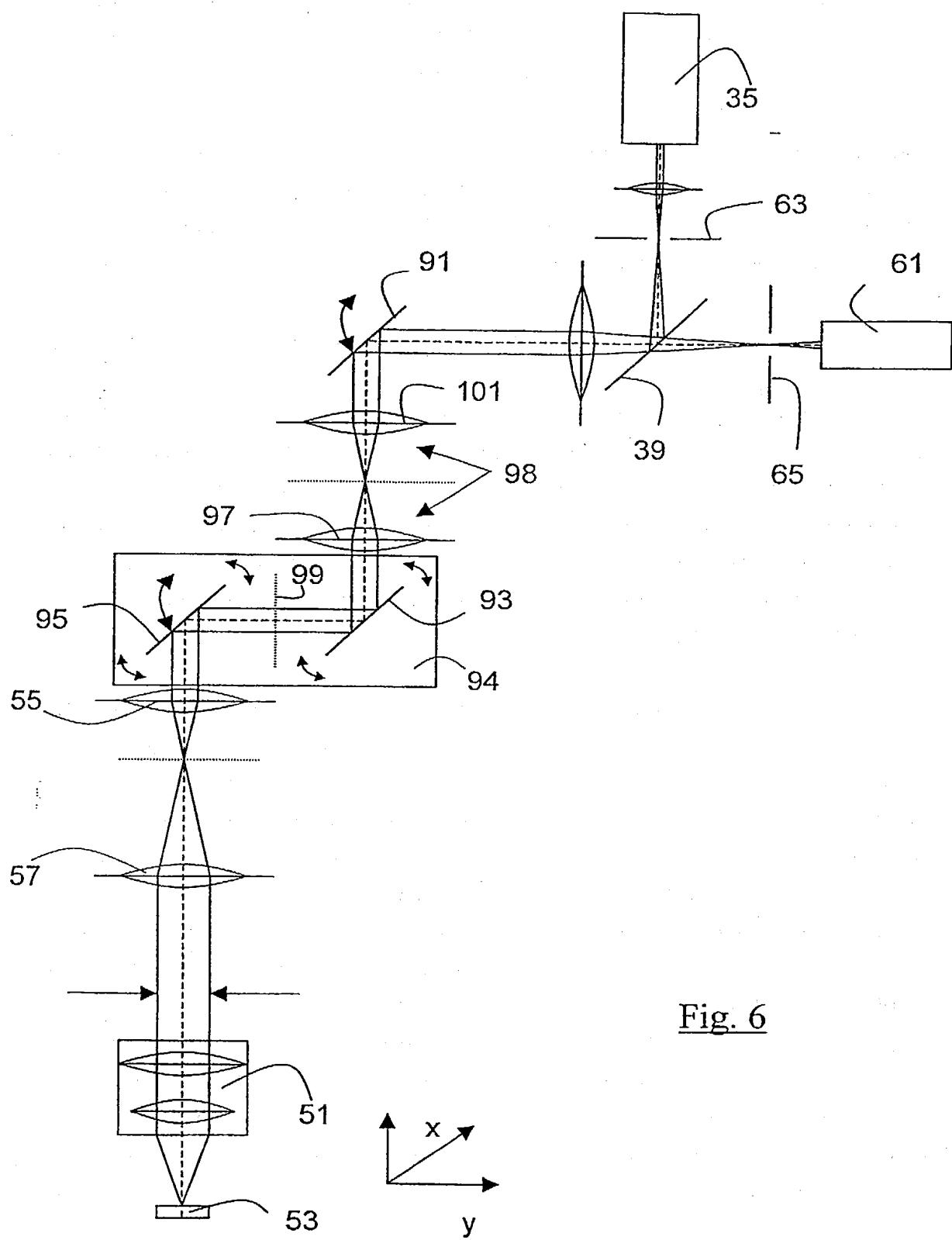


Fig. 5



**METHOD FOR BEAM CONTROL IN A SCANNING MICROSCOPE, ARRANGEMENT
FOR BEAM CONTROL IN A SCANNING MICROSCOPE, AND SCANNING
MICROSCOPE**

The invention concerns a method for scanning individual regions in scanning microscopy, the regions of interest being distributed over the entire image field. It is possible to switch rapidly between the individual regions of interest while maintaining the scanning motion. The scanning motion can be accomplished by way of a suitable motion of a scanning mirror.

The invention further concerns an arrangement for beam control in scanning microscopy.

In addition, the invention concerns a scanning microscope that comprises an arrangement for beam control which makes it possible to switch rapidly between the individual regions of interest while maintaining the scanning motion. The scanning microscope can also be configured as a confocal microscope. In particular, in the scanning microscope a light beam produced by an illumination system is guided over a specimen with the interposition of several optical means, and it contains at least one detector that, by way of the several optical means, detects a light proceeding from the specimen.

In scanning microscopy, a sample is illuminated with a light beam in order to observe the reflected or fluorescent light emitted from the sample. The focus of the illuminating light beam is moved in a specimen plane by means of a controllable beam deflection device, generally by tilting two mirrors, the deflection axes usually being at right angles to one another so that one mirror deflects in the X and the other in the Y direction. The tilting of the mirrors is brought about, for example, using galvanometer positioning elements; both fast resonant galvanometer positioning elements and slower (more accurate) non-resonant ones are used. In order to scan a sample in the specimen plane, it is important that the rotation axes of the mirror lie in or at least near a plane, also referred to as the Fourier plane, conjugated with the focal plane. One possible beam deflection device that meets the requirements for telecentric imaging is known, for example, from DE 196 54 210. The power level of the light coming from the specimen is measured as a function of the position of the scanning beam. Usually the positioning elements are equipped with sensors for ascertaining the present mirror position.

In confocal scanning microscopy specifically, a specimen is scanned in three dimensions with the focus of a light beam.

A confocal scanning microscope generally comprises a light source, a focusing optical system with which the light of the source is focused onto a pinhole (called the excitation stop), a beam splitter, a beam deflection device for beam control, a microscope optical system, a detection stop, and the detectors for detecting the detected or fluorescent light. The illuminating light is coupled in via a beam splitter. The fluorescent or reflected light coming from the specimen arrives via the beam deflection device back at the beam splitter, passes through it, and is then focused on the detection stop behind which the detectors are located. Detected light that does not derive directly from the focus region takes a different light path and does not pass through the detection stop, thus yielding a point datum that results, by sequential scanning of the specimen, in a three-dimensional image. A three-dimensional image is usually obtained by acquiring image data in layers.

Ideally, the track of the scanning light beam on or in the specimen describes a meander that fills the entire image field (scanning one line in the X direction at a constant Y position, then stopping the X scan and slewing by Y displacement to the next line to be scanned, then scanning that line in the negative X direction at constant Y position, etc.). At high beam deflection speeds, deviations from the ideal track occur because of the inertia of the deflecting moving parts, for example the galvanometer shaft and the mirrors. At usable scanning rates (> 100 Hz) the scanning track of the light beam actually describes a sine curve, which in fact necessitates a correction of the deviations from the ideal situation resulting therefrom.

The power level of the light coming from the specimen is measured at fixed time intervals during the scanning operation, and thus scanned one grid point at a time. The reading must be unequivocally associated with the pertinent scan position so that an image can be generated from the measured data. Advantageously this is done by also continuously measuring the status data of the adjusting elements of the beam deflection device, or (although this is less accurate) by directly using the reference control data of the beam deflection device.

In some microscopy applications the user is interested only in information about individual regions within the image field, while the surrounding sample regions are not of interest. The regions of interest should moreover be scanned as quickly as possible in succession.

Known arrangements offer only a limited capability for scanning individual sample regions of interest. Scanning the entire image field and subsequently selecting the data of the regions of interest is feasible, if at all, only to a limited extent given the required rapid sequential acquisition of information about the regions of interest.

The approach of sequentially scanning the individual regions of interest is better. It is possible in principle to activate the beam deflection device in such a way that each of the regions of interest is separately scanned, for example, in meander fashion, and the surrounding regions that are not of interest are not scanned. This procedure is possible, however, only if the beam deflection device allows the scanning light beam to be specifically controlled and specifically directed onto individual points in the image field.

This is not possible when using resonantly operating beam deflection devices which are based, for example, on the use of resonant galvanometers or micromirrors, because these beam deflection devices operate exclusively at the particular resonant frequency dictated by their design. It is not possible to "park" the light beam in one region of the image field. Difficulties also occur with rapidly deflecting non-resonantly operating beam deflection devices in terms of the positionability that can be achieved, since the positioning elements react to an activation signal in delayed fashion because of their inertia.

It is therefore the object of the invention to describe a method for scanning microscopic preparations with a light beam that solves the problem described above.

This object is achieved by way of a method that comprises the following steps:

- acquiring a preview image;
- marking at least one region of interest in the preview image;
- displacing the scan field onto the region of interest by means of a first beam deflection device; and
- acquiring an image by meander-shaped scanning of the region of interest with a second beam deflection device.

What has been recognized according to the present invention is that it is not necessary to forgo the use of fast or resonant beam deflection devices if the scan field swept by the first beam deflection device is displaced within the image field onto the regions of interest with the aid of a further suitable beam deflection device that allows exact positioning.

A further object of the invention is to create an arrangement for beam control in a scanning microscope which makes it possible to switch rapidly among several regions of interest and, in that context, to collect information from regions of interest based on a consistent pattern.

This object is achieved by an arrangement for beam control in a scanning microscope, the scanning microscope comprising a microscope optical system and means for acquiring and displaying a preview image. The arrangement is characterized in that

- means for marking at least one region of interest in the preview image;
- a first beam deflection device for displacing the scan field onto the region of interest; and
- a second beam deflection device for meander-shaped scanning within the scan field

are provided.

In a particular embodiment, according to the present invention an imaging optical system is provided between the beam deflection devices in order to guarantee the principle of telecentric scanning.

A further object of the invention is to create a scanning microscope that makes possible rapid sequential scanning of sample regions of interest.

This object is achieved by a scanning microscope which is characterized in that

- means for marking at least one region of interest in the preview image;
- a first beam deflection device for displacing the scan field onto the region of interest; and
- a second beam deflection device for meander-shaped scanning within the scan field

are provided.

The invention has the advantage that it is not necessary to forgo the use of fast or resonant beam deflection devices if the scan field swept by the first beam deflection device is

displaced within the image field onto the regions of interest with the aid of a second suitable beam deflection device that allows exact positioning. Ideally, the track of the scanning light beam on or in the specimen describes a rectangular curve (scan one line in the X direction at a constant Y position, then stop the X scan and slew by Y displacement to the next line to be scanned, then scan that line in the negative X direction at constant Y position, etc.). At increasingly high deflection speeds, the scanning track deviates more and more from the rectangular shape. This phenomenon is attributable substantially to the inertia of the moving elements. With rapid scanning, the scanning track is more similar to a sine curve. In this context, the scan field is swept by the light beam in such a way that the reversing points of the sinusoidal track lie outside the region of interest. The scanning light beam thus describes approximately straight tracks on the region of interest.

The subject matter of the invention is depicted schematically in the drawings and will be described below with reference to the Figures, in which:

FIG. 1 schematically depicts selection of the regions of interest;

FIG. 2 schematically depicts the shape of the scanning track;

FIG. 3 shows a scanning microscope having the arrangement according to the present invention;

FIG. 4 shows a scanning microscope according to the present invention;

FIG. 5 shows a further embodiment of the scanning microscope according to the present invention; and

FIG. 6 shows a further embodiment of the scanning microscope having an arrangement according to the present invention.

FIG. 1 shows a PC 1 having a monitor 3 and a cursor controller 5. In one embodiment, cursor controller 5 is a computer mouse. Further possibilities for a cursor controller 5 are, for example, a joystick, a trackball, or any other conceivable cursor control device. A preview image 7 of the entire image field 19 is displayed on the monitor. Using the computer mouse, image areas 11, 13 of regions of interest 27, 29 are marked with cursor 9. The marking is displayed in the form of bordering lines 15, 17.

FIG. 2 schematically depicts the shape of the scanning track, which is made up of partial scanning tracks 21, 23, and 25. Partial scanning tracks 21 and 25 are caused by a fast, resonant beam deflection device, whereas partial scanning track 23 is caused by a beam deflection device for specific positioning. Region of interest 27 is traversed by partial scanning track 21, whereas region of interest 29 is traversed by partial scanning track 25. Scan fields 31 and 33 are consequently scanned sequentially. Partial scanning track 23 is swept because of the displacement of scan fields 31, 33 using the second beam deflection device for specific positioning.

FIG. 3 schematically shows a confocal scanning microscope. Light beam 37 coming from an illumination system 35 is reflected from a beam splitter 39 via deflection mirror 41 to first beam deflection device 43 for specific positioning, which contains a gimbal-suspended scanning mirror (not shown) and serves to displace the scan field in a plane perpendicular to the illumination direction. By way of an intermediate image using an imaging optical system 47 that generates an intermediate focal plane 45, light beam 37 arrives at second beam deflection device 49 which is embodied as a K-mirror (see DE 196 54 210) and contains two resonant galvanometers (not shown). Second beam deflection device 49 provides meander-shaped scanning within the scan field. The size of a scan field can be varied by defining the deflection angle of the galvanometers. Light beam 37 is consequently guided over or through sample 53 via scanning lens 55, optical system 57 and through microscope optical system 51. In the case of non-transparent specimens 15, light beam 37 is guided over the specimen surface. In the case of biological samples (preparations) or transparent samples, light beam 37 can also be guided through sample 53. This means that different focal planes of sample 53 are successively scanned by the focus of light beam 37. Detected light 59 proceeding from sample 53 arrives, on the reverse light path via first and second beam deflection devices 43, 49, back at beam splitter 39, passes through it, and is then detected with detector 61. Detector 61 comprises a photomultiplier that converts the detected light data into electrical signals. Subsequent assembly of the signals and allocation to the respective scan positions then yields a three-dimensional image of region of interest 27, 29 of sample 53. Illumination pinhole 63 and detection pinhole 65 that are usually provided in a confocal scanning microscope are shown schematically for the sake of completeness. Certain optical elements for guiding and shaping the light beams are omitted, however, for better clarity; these are sufficiently known to those skilled in this art.

FIG. 4 shows a confocal scanning microscope having a first beam deflection device 67 for specific position. Second beam deflection device 72, which is constituted by a first and a second beam deflection module 71 and 75, is provided for rapid scanning of the scan field. The two beam deflection modules 71 and 75 can, for example, each contain a resonantly oscillating micromirror. Imaging optical systems 69, 73 are provided between first and second beam deflection devices 67 and first and second beam deflection modules 71 and 75 to maintain the telecentric principle.

FIG. 5 shows a confocal scanning microscope having a total of four beam deflection modules 77, 79, 81, 83 that are distributed respectively among two beam deflection devices 78 and 82. Beam deflection modules 77 and 81 deflect in the X direction, whereas beam deflection modules 79 and 83 deflect in the Y direction. Beam deflection device 78 serves to position scan field 31, 33. It contains, for example, galvanometers that operate non-resonantly. Beam deflection device 82 operates resonantly, and serves to scan scan field 31, 33 in meander-shaped fashion. Beam deflection modules 77, 79 and 81, 83 are arranged close to planes 89 and 87, respectively, that are conjugated with the focal plane of microscope optical system 51. Imaging optical system 85 is provided to maintain the telecentric principle.

FIG. 6 shows a further exemplary embodiment of a confocal scanning microscope. Here it is the gimbal-mounted mirror 91, driven in non-resonant fashion, that serves to position scan field 31, 33. In order to maintain the telecentric scanning principle, mirror 93 that deflects in the Y direction and is driven by a resonant galvanometer, and mirror 95 that deflects in the X direction and is driven by a resonant galvanometer, are arranged close to plane 99 conjugated with the focal plane of microscope optical system 51. Imaging optical system 98, which is made up of optical systems 97 and 101 and generates at the rotation point of mirror 91 a further plane 99 conjugated with the focal plane of microscope optical system 51, is located between mirrors 93 and 91. In this exemplary embodiment, second beam deflection device 94 comprises mirrors 93 and 95.

First beam deflection device 43, 67, 78 and/or second beam deflection device 49, 72, 82, 94 contain, inter alia, a gimbal-mounted mirror or a micromirror or an acoustooptical deflector or a galvanometer mirror or a resonantly oscillating mirror system.

The present invention was described with reference to a particular exemplary embodiment. It is, however, self-evident that changes and modifications can be made without thereby leaving the range of protection of the claims recited hereinafter.

CLAIMS

1. A method for beam control in a scanning microscope, characterized by the following steps:
 - acquiring a preview image;
 - marking at least one region of interest in the preview image;
 - displacing the scan field onto the region of interest by means of a first beam deflection device; and
 - acquiring an image by meander-shaped scanning of the region of interest with a second beam deflection device.
2. A method as claimed in claim 1, characterized by a further step in which the first beam deflection device transfers the meander-shaped scanning of a region of interest into the meander-shaped scanning of a further region of interest.
3. A method as claimed in claim 2, wherein the meander-shaped scanning of the regions of interest proceeds in accordance with a defined sequence and acquires information from the regions of interest.
4. A method as claimed in claim 3, wherein the sequence of the meander-shaped scanning of the regions of interest is user-defined.
5. A method as claimed in claim 3, wherein the sequence of the meander-shaped scanning of the regions of interest is performed automatically on the basis of the information obtained from the regions of interest.
6. An arrangement for beam control in scanning microscopy, the scanning microscope comprising means for acquiring and displaying a preview image and a microscope optical system, wherein
 - means for marking at least one region of interest in the preview image;
 - a first beam deflection device for displacing the scan field onto the region of interest; and
 - a second beam deflection device for meander-shaped scanning within the scan fieldare provided.

7. An arrangement for beam control as claimed in claim 6, wherein the first beam deflection device transfers the meander-shaped scanning of a region of interest into the meander-shaped scanning of a further region of interest.
8. An arrangement as claimed in claim 6, wherein the second beam deflection device comprises two beam deflection modules.
9. An arrangement as claimed in claim 6 or 8, wherein the first beam deflection device comprises two beam deflection modules.
10. An arrangement as claimed in claim 6, wherein the first beam deflection device and/or second beam deflection device contains or contain gimbal-mounted mirrors or a micromirror or an acousto-optical deflector or a galvanometer mirror or a resonantly oscillating mirror system.
11. An arrangement as claimed in claim 6, wherein the first beam deflection device is arranged substantially in the vicinity of a plane conjugate with the focal plane of the microscope optical system.
12. An arrangement as claimed in claim 6, wherein the second beam deflection device is arranged substantially in the vicinity of a plane conjugate with the focal plane of the microscope optical system.
13. An arrangement as claimed in claim 11 or 12, wherein an imaging optical system is provided between the first beam deflection device and second beam deflection device.
14. An arrangement as claimed in claim 13, wherein a relay optical system is provided between the first beam deflection device and second beam deflection device.
15. A scanning microscope having an arrangement for beam control, the scanning microscope comprising means for acquiring and displaying a preview image and a microscope optical system, wherein
 - means for marking at least one region of interest in the preview image;
 - a first beam deflection device for displacing the scan field onto the region of interest; and

- a second beam deflection device for meander-shaped scanning within the scan field

are provided.

16. A scanning microscope as claimed in claim 15, wherein the first beam deflection device transfers the meander-shaped scanning of a region of interest into the meander-shaped scanning of a further region of interest.
17. A scanning microscope as claimed in claim 15, wherein the second beam deflection device comprises two beam deflection modules.
18. A scanning microscope as claimed in claim 15 or 17, wherein the first beam deflection device comprises two beam deflection modules.
19. A scanning microscope as claimed in claim 15, wherein the first beam deflection device and/or second beam deflection device contains or contain gimbal-mounted mirrors or a micromirror or an acousto-optical deflector or a galvanometer mirror or a resonantly oscillating mirror system.
20. A scanning microscope as claimed in claim 15, wherein the first beam deflection device is arranged substantially in the vicinity of a plane conjugate with the focal plane of the microscope optical system.
21. A scanning microscope as claimed in claim 15, wherein the second beam deflection device is arranged substantially in the vicinity of a plane conjugate with the focal plane of the microscope optical system.
22. A scanning microscope as claimed in claim 20 or 21, wherein an imaging optical system is provided between the first beam deflection device and second beam deflection device.
23. A scanning microscope as claimed in claim 22, wherein a relay optical system is provided between the first beam deflection device and second beam deflection device.



Application No: GB 0123392.3
Claims searched: 1, 6 and 15

12

Examiner: Rebecca Villis
Date of search: 27 March 2002

Patents Act 1977
Search Report under Section 17

Databases searched:

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:

UK Cl (Ed.T): G2J (JB7WX); H4F (FAAL), (FCCC), (FCCY), (FJB), (FJC)

Int Cl (Ed.7):

Other: Online: EPODOC, WPI, PAJ

Documents considered to be relevant:

Category	Identity of document and relevant passage		Relevant to claims
A,&	DE 19754647	(HITACHI)	
A	JP 2309881	(SAKAMOTO)	
A,&P	US 6225628	(HITACHI)	
A	US 4691103	(PHILIPS)	

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application.

